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ABSTRACT

Background: According to cross-sectional and acute experimental evidence, reducing sitting time should improve cardio-metabolic health risk biomarkers. Furthermore, the improvements obtained may depend on whether sitting is replaced with standing or ambulatory activities. Based on data from the *Stand Up Victoria* multi-component workplace intervention, we examined this issue using compositional data analysis — a method that can examine and compare all activity changes simultaneously.

Methods: Participants receiving the intervention (n=136 ≥0.6 full-time equivalent desk-based workers, 65% women, mean±SD age=44.6 ±9.1 years from seven worksites) were asked to improve whole-of-day activity by standing up, sitting less and moving more. Their changes in the composition of daily waking hours (activPAL-assessed sitting, standing, stepping) were quantified, then tested for associations with concurrent changes in cardio-metabolic risk (CMR) scores and 14 biomarkers concerning body composition, glucose, insulin and lipid metabolism. Analyses were by mixed models, accounting for clustering (3 months, n=105–120; 12 months, n=80–97).

Results: Sitting reduction was significantly ($p<0.05$) associated only with lower systolic blood pressure at three months, and with CMR scores, weight, body fat, waist circumference, diastolic blood pressure, and fasting triglycerides, total/HDL cholesterol and insulin at 12 months. Significant differences between standing and stepping were only observed for systolic blood pressure and insulin; both favored stepping. However, replacing sitting with standing was significantly associated only with improvements in CMR scores, while replacing sitting with stepping was significantly associated with CMR scores and six biomarkers.

Conclusions: Improvements in several cardio-metabolic health risk biomarkers were significantly associated with sitting reductions that occurred in a workplace intervention. The greatest degree and/or widest range of cardio-metabolic benefits appeared to occur with long-term changes, and when increasing ambulatory activities.

Keywords: sedentary; compositional data analysis (CoDA); ambulation; intervention; biomarkers

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INTRODUCTION

Increased risk of developing cardiovascular disease and diabetes (1), and elevated biomarkers of risk for these chronic diseases (2), have been observed with high volumes of sitting time, and especially sitting time accrued in a prolonged, continuous manner. Supporting the epidemiological evidence, laboratory studies have shown acute benefits to glucose, insulin, and lipid metabolism of interspersing long periods of sitting with even small amounts of activity (3-5). Accordingly, interventions to reduce sitting, especially in the workplace — a key setting for addressing prolonged sitting time — have been advocated as a public-health strategy (6, 7). In particular, sit-stand workstations have emerged as effective tools in multi-component workplace sitting interventions (8) as their usage reduces sitting time by large volumes.

By contrast with the clear evidence that such interventions can reduce sitting time, the evidence concerning whether they are likely to impart non-acute benefits to cardio-metabolic health is less clear, especially when sitting is primarily replaced with standing. Workplace sitting-reduction interventions that primarily increase standing (e.g., through installation of sit-stand desks) have shown benefits concerning lipid and glucose biomarkers, but inconsistently (9-11). Notably, thus far, only the sitting-reduction interventions that have increased stepping (e.g., by use of treadmill desks) have shown significant benefits to body weight or body composition (12, 13). The short-term evaluations and insufficient sample sizes of most studies may explain the mixed findings. However, it is also possible that the potential cardio-metabolic benefits of reducing sitting in an intervention are inherently variable because participants can make a plethora of different behavior changes when reducing sitting. Potentially relevant considerations include the volume

of sitting reduction, the activities replacing sitting (e.g., standing versus ambulatory activities), and any compensatory activity changes that may or may not occur (14).

Recently, compositional data analysis (CoDA) has been used to simultaneously examine all activities occupying a 24-hour day and test them in relation to cardio-metabolic biomarkers (15). The study findings revealed that some biomarkers, notably those pertaining to glucose metabolism, improve significantly when increasing light activity at the expense of sedentary time (15). Importantly, CoDA is a valid method for examining data that sum to a fixed total, such as 24 hours (15) and it can be applied to evaluate all of the changes in activity that occur during an intervention simultaneously, and test these in relation to changes in cardio-metabolic biomarkers. To our knowledge, CoDA has not been applied in this context, nor to the examination of standing as a separate component from ambulatory light activities. Using CoDA, we therefore examined the associations of short- and long-term (3- and 12-month) changes in daily time use with concurrent changes in cardio-metabolic biomarkers, within participants receiving the *Stand Up Victoria* intervention.

METHODS

The *Stand Up Victoria* cluster-randomized trial was registered with the Australian New Zealand Clinical Trials register (ACTRN12611000742976). The Alfred Health Human Ethics Committee (Melbourne, Australia) granted ethical approval. Participants provided written consent. The study was conducted in accordance with the CONSORT guidelines for cluster-randomized trials (http://www.consort-statement.org/). Details are published elsewhere concerning the study

protocol (16), the measures used, development and pilot testing (10, 17), evaluation of the main activity outcomes (18) and the secondary cardio-metabolic biomarker outcomes(19).

Setting and participants

Teams from study worksites that were at least one kilometre apart were identified and recruited from a single organization, then were randomized to the intervention (n=7 sites, n=136 workers) or control (n=7 sites, n=95 workers) condition. Eligibility criteria for individual participants in the selected teams were: aged 18–65 years; not pregnant; ambulatory; speaks English; capable of standing or sitting for ≥10 minutes continuously; and, working ≥0.6 full time equivalent with designated access to a telephone, internet, and desk. Participants and study staff were not blinded to group allocation. The present study evaluates only the intervention participants.

Intervention

The *Stand Up Victoria* intervention consisted of organizational support (senior management support, a team champion who sent emails containing the intervention messages); environmental modification (sit-stand workstations); and, individual health coaching (including goal setting and tracking). The intervention was tapered over 12 months with intensive components (e.g., health coaching, team champion intervention) ceasing after 3 months. It primarily targeted reductions in workplace sitting time, especially sitting accrued for ≥30 minutes at a time continuously. The main message was to "Stand Up, Sit Less, Move More". The intervention encouraged participants to replace part of their sitting across the entire day with standing and stepping, by standing at their workstation for at least an hour a day, and by using a variety of self-selected strategies, which might target standing, stepping or both. Evaluation of the study's activity

outcomes previously revealed that, net of control, the intervention on average produced moderately large effects on reduced sitting and increased standing (≈ 80 min/day at 3-months and ≈ 40 min/day at 12-months) with no significant effect on stepping (-6 min/day at 12-months) (18). These effects were established across the entire waking day (i.e., at work and outside of work, considering the entire week rather than just workdays). Cardio-metabolic biomarker outcomes, net of control, showed a significant improvement in overall cardio-metabolic risk and fasting glucose at 12 months, and non-significant (but typically favorable) effects on the other biomarkers (19).

Data collection and measures

Measurements were at baseline, three months into the intervention (upon completion of the individual-level health coaching and champion emails) and at 12 months, and included an onsite assessment of biomarkers and an activity monitoring assessment. Further participant characteristics were assessed using ~~O~~online questionnaires (LimeService: www.limeservice.com) ~~assessed most other participant characteristics~~.

Cardio-metabolic biomarker outcomes

The collection of these biomarkers is described in detail elsewhere(19), along with their changes over the course of the intervention. The cardio-metabolic biomarkers examined were: systolic blood pressure, diastolic blood pressure, weight, fat mass (kg, % of bodyweight), waist circumference, fasting triglycerides, high-density lipoprotein (HDL)- and low density lipoprotein (LDL)- cholesterol, total/HDL cholesterol ratio, glucose, insulin, insulin sensitivity (%S) and steady state beta cell function (%B) as calculated using the homeostatic model assessment

(HOMA2) online calculator (https://www.dtu.ox.ac.uk/homacalculator/) version 2.2.3 and an overall cardio-metabolic risk (CMR) score. CMR scores (20) were calculated by first log10 transforming and normalizing (mean/SD) the relevant biomarkers, then by taking a weighted average of their values:1/5·waist circumference + 1/5·triglycerides -+ 1/5·HDL-cholesterol + 1/5·fasting glucose + 1/5· mean of systolic and diastolic blood pressure. Changes in the biomarkers were calculated as follow up score minus baseline score.

Activity measures

Activity was measured by the highly accurate (21) and responsive (22) activPAL3^{TM} activity monitor (PAL Technologies Limited, Glasgow, UK; minimum version 6.3.0). The waterproofed monitor was secured onto the right anterior thigh with a hypoallergenic patch at the onsite assessment. Each participant was asked to wear the monitor continuously (24 h/day) for the following seven days, and to record the following times daily in a diary: starting and finishing work; waking up; going to sleep ("lights out"); removing and re-attaching the monitor. Monitor data were processed as reported in the primary outcomes paper (18). Though daily activities can be classified in many ways, we subdivided time use by activity classifications consistent with the intervention and measurement tool: sitting, standing, and stepping (during waking hours, while wearing the monitor) and "other" time (non-wear time and time in bed).

Statistical analyses

Analyses were performed in STATA version 13 (STATACorp, College Station, Texas, US) and R version 3.3.0, using the packages "compositions" ("acomp" framework) "nlme" and "lsmeans". Statistical significance was set at $p<0.05$, two-tailed. Missing data were excluded.

Quantifying activity and activity change compositionally

We used compositional methods, which have been outlined as applied to cross-sectional physical activity and sedentary behavior data by Chastin et al (15). The total 24-hour day was divided across four activities (stepping, standing, sitting, "other"). Sleep, other time in bed and non-wear time comprised "other" time (i.e., 24 hours minus monitored waking hours). CoDA's property of "sub-compositional coherence" means that the exclusion of irrelevant activities does not adversely affect results (23). The analysis includes only the sub-composition of activities that comprise waking hours (stepping, standing, sitting); i.e., the composition of waking hours. "Other" time was excluded in order to reduce the number of dimensions and provide efficient estimates. This decision seemed to be reasonable since the "other" time was not targeted by the intervention and did not change much over time at the group level or for individuals. At baseline, three months, and 12 months, compositions were calculated using the R function "acomp". No method was required to address the problem of zero time use, as all participants spent some time in every time-use category at each assessment. Compositional changes $[\mathrm{Step}_{\Delta}, \mathrm{Stand}_{\Delta}, \mathrm{Sit}_{\Delta}]$ were then measured by Aitchison's perturbation method (23, 24). The ratios of each component in the composition or sub-composition, such as $\left[\frac{\mathrm{Step}_{12M}}{Step_{BL}}, \frac{\mathrm{Stand}_{12M}}{Stand_{BL}}, \frac{\mathrm{Sit}_{12M}}{Sit_{BL}}\right]$ for 12-month changes from baseline, were calculated and were then divided by the sum total of these ratios. An equal composition of these three activities at baseline and follow up would result in a compositional change of [1/3,1/3,1/3]. Compositional changes were plotted as ternary diagrams (Figure 1), with ~~key~~ some guide values marked: no change; average sitting reduces by 1 h/16h day replaced with

either all stepping, all standing, or half of each; and, the average sitting reduces by 2 h/16h day replaced entirely with standing.

Quantifying associations of activity changes with biomarker changes

The associations of activity changes with biomarker changes were examined as mixed models ("lme" function), with a random intercept for cluster, and fixed effects for changes in the activity composition [$\mathrm{Step}_{\Delta}, \mathrm{Stand}_{\Delta}, \mathrm{Sit}_{\Delta}$]. Short- and long-term changes were examined separately. Briefly, we used an isometric log-ratio transformation (i.e., "ilr" function) to measure the compositional change as two parameters (z1 and z2). These parameters are orthogonal and can therefore be safely included together as independent variables in the mixed models (15, 23). The isometric log-ratio transformation can be performed from a number of perspectives. The primary perspective we used allows for the effect of a decrease in the parameter z1 on biomarkers to indicate the effects of making sitting a smaller proportion of the waking day. These effects are estimated while controlling for shifts in the remaining non-sitting time between standing and stepping, the effect of which is measured as the parameter z2. The transformation was as follows:

$$z1_{Sit\ vs\ stand\ \&\ step} = \sqrt{\frac{2}{3}} \ln\left(\frac{Sit_{\Delta}}{\sqrt{Stand_{\Delta} \times Step_{\Delta}}}\right) \ [Eq.1]$$

$$z2_{Stand\ vs\ step} = \sqrt{\frac{1}{2}} \ln\left(\frac{Stand_{\Delta}}{Step_{\Delta}}\right) \ [Eq.2]$$

In addition, we presented selected estimates for the z2 parameter calculated from different perspectives that indicate the effects of shifts in non-stepping time between sitting and standing

(more standing less sitting), and the shifts in non-standing time between sitting and stepping (more stepping less sitting). Although the direction and significance of the parameters can be used to understand the findings, the clinical relevance of the coefficients is not straightforward. Estimates were presented partially standardized, with biomarker changes all expressed as a number of baseline standard deviations, so that the relative effects on the different biomarkers can be compared. To better understand the results, tertiles of predicted improvement (most improved/least worsened to least improved/most worsened) were plotted across changes in the composition that participants made (as presented in Figure 2). Also, to better indicate effect sizes, the predicted mean improvement was calculated across a range of standing and stepping changes in the composition that culminate in reducing sitting to recommended levels of 50% (25). Consistent with the use of CoDA methods, our analyses did not adjust for total waking hours (or wear time). Instead, a sensitivity analysis using the composition of all ~~waking~~ 24 hours was conducted to verify that excluding changes in "other" time was reasonable (and by implication that ignoring the total amount of waking hours was reasonable).

RESULTS

Baseline characteristics of intervention participants are shown in Supplemental Table 1. Relevant data on short- and long-term changes were available from 105–120 participants (77–88%) and 80–97 (59–71%), respectively. Generally, those who provided data were similar to those who dropped out, with the exception being that more women than men dropped out during the intervention, which shifted anthropometric biomarkers in directions expected for a group containing more males.

Activity composition

Activity outcomes have been reported previously (18). Considering activity as a composition of daily time use, the intervention group's daily activity was very high in sitting, low in standing and very low in stepping both at baseline [65.4%, 24.1%, 10.5%] and to a lesser extent at 12 months [60.4%, 29.5%, 10.1%] (Supplemental Figure 1), corresponding to a mean 12-month change of [0.30, 0.39, 0.31]. Figure 1 is a ternary plot of the 12-month changes, with each corner indicating a complete change towards that activity (from 0% to 100% of waking hours) and with the centre indicating no change. Individual changes made by participants were highly variable. The mean change in the composition was statistically significant (with the 95% confidence region excluding no change) and was very close to the point indicating a drop in mean baseline sitting of 1 hour/16 hours awake, when sitting is replaced exclusively with standing.

Changes in the activity composition with changes in biomarkers

Three-month sitting reductions were significantly associated only with changes in systolic blood pressure ($p=0.039$), with the direction of associations indicating sitting reduction to be beneficial (Tables 1–2). Long-term (12-month) sitting reductions were significantly associated with improvements in CMR, triglycerides, total/HDL cholesterol ratio, diastolic blood pressure, weight and body fat, waist circumference and insulin, and had a borderline significant ($p=0.063$) association with improved insulin sensitivity (Tables 3–4).

In terms of the forms of sitting reductions associated with biomarker changes, overall CMR scores improved significantly with sitting-standing substitutions ($p=0.031$) and with sitting-stepping substitutions ($p=0.028$) without a statistically significant difference between standing

and stepping (p=0.240). By contrast, for fasting insulin and insulin sensitivity (HOMA-S), stepping was significantly better than standing as a sitting replacement (p=0.006 and 0.032). No significant effect on these biomarkers was seen of replacing sitting with standing (p=0.889 and 0.943) whereas replacing sitting with stepping was associated with significant benefit (p=0.006 and 0.029). Figure 2 displays the results graphically. CMR improvements were seen when reducing the contribution of sitting to the overall waking day. At some levels of sitting change, there was patterning whereby more CMR improvement was seen when the remaining time use was shifted more towards stepping rather than standing (i.e., from left to right across the graph), but this was not evident with the largest sitting reductions. All of the participants in the most improved tertile of CMR had made sitting reductions. Figure 2b shows that the degree of improvement that occurred at all levels of sitting change appeared dependent on how much of the remaining (non-sitting) time use shifted towards stepping (most beneficial) versus standing.

For the other outcomes that had significantly improved with long-term sitting reduction (i.e., triglycerides, total/HDL cholesterol, diastolic blood pressure, weight, body fat (kg and %) and waist circumference), it was not clear whether or not these improvements depended on sitting being replaced with ambulatory activities. Suggestive that either standing or ambulation can improve these outcomes, there was no significant difference whether sitting was replaced with standing or stepping. However, the effects on these outcomes observed for replacing sitting with standing did not reach statistical significance, while replacing sitting with stepping was significantly associated with improved total/HDL cholesterol ratio (p=0.045), diastolic blood pressure (p=0.027), and fat mass (kg and %, p=0.034 and 0.022). In addition to statistical significance, the direction of the results, and the patterning of biomarker changes across activity

as plotted in Supplemental Figures 2–5, are informative. These were consistent with these biomarkers improving somewhat by substituting sitting with standing and improving slightly more by substituting sitting with stepping. Supplemental Table 2 shows the estimated mean 12-month changes in cardio-metabolic outcomes when reducing baseline mean sitting (65.4%) to desirable levels (50%) via various replacement strategies. Moderate to strong improvements (0.5–0.8 SD) were seen for many outcomes but only with substantial increases in ambulation. In order to see a small improvement in mean biomarkers (0.2 SD), only a small percentage of the sitting reduction needed be achieved by increasing ambulatory activities for lipids and blood pressure (20% or less), for insulin (21%) and for some of the adiposity indicators (waist circumference and body fat percentage). The requirement for ambulation was higher for the other outcomes, ranging from 30% to 68% of the sitting replacement.

Changes in the amount of "other" time relative to sitting standing and stepping were only significantly associated with systolic blood pressure at 12 months, and triglycerides, HDL cholesterol and HOMA-S at three months (Supplemental Table 3). For all these outcomes, the conclusions concerning reducing sitting relative to standing and stepping, and shifts between standing and stepping were no different whether examining all hours or only waking hours.

DISCUSSION

Previously, we showed the *Stand Up Victoria* workplace sitting-reduction intervention predominantly reduced sitting by increasing standing (18), and was effective in the long term for improving fasting glucose and an overall CMR score, net of control (19). The present study extends from these findings to understand how the various activity changes that intervention

247 participants made were associated with concurrent biomarker changes, using a novel application
248 of compositional analysis. We found that sitting reduction was associated with significant
249 improvements in the biomarkers of cardiovascular and metabolic health across all of the areas
250 examined (glucose and insulin metabolism, lipid metabolism, blood pressure, body composition).
251 To varying degrees, the various benefits appeared to depend on the type of sitting reduction (i.e.,
252 whether sitting was replaced with standing or with stepping).
253
254 Both the previously reported outcomes of the workplace sitting intervention (19) and the present
255 findings may indicate the need for long-term intervention to improve biomarkers via sitting
256 reduction. We saw many significant associations of activity changes with biomarker changes
257 over a 12-month timeframe, and very few over a three-month period. While this could be a
258 chance finding, it could also reflect a physiological requirement for long-term behavior change in
259 order to improve these biomarkers. Either way, it appears prudent to investigate long-term
260 effects rather than infer them from short-term interventions, where benefits may be missed.
261
262 Our CMR findings showed that cardio-metabolic biomarker improvement can occur when
263 replacing sitting time with non-ambulatory activities. However, findings for the individual
264 biomarkers suggested the degree and/or range of cardio-metabolic biomarker improvements may
265 be greater when replacing sitting with ambulation than with standing. Fasting insulin and
266 HOMA-S improved significantly more by replacing sitting with stepping than with standing.
267 Some of the findings showed seemingly conflicting results whereby standing was neither
268 significantly beneficial, nor significantly inferior to stepping. This apparent conflict is potentially
269 explained by the study's sample size providing insufficient precision to distinguish standing from

either sitting or stepping, with standing having an impact that was more beneficial than sitting but less beneficial than stepping. Larger RCTs or meta-analyses may yield further insights as to potential benefits of replacing sitting with standing within field-based sitting-reduction interventions. Cross-sectionally, in isotemporal analyses, reallocating time use away from sitting towards additional standing has shown significant beneficial associations with triglycerides, HDL cholesterol, total/HDL cholesterol ratio and fasting glucose though not with weight or waist circumference (26). In addition to the outcomes that appear important from the existing literature, our findings suggest that key biomarkers that might be important to collect when evaluating interventions similar to Stand Up Victoria are: those comprising CMR scores; those showing the greatest response to substituting sitting specifically with standing (i.e., waist circumference, fasting glucose, triglycerides and diastolic blood pressure, whose coefficients for sitting versus standing were largest at ≈0.3 to 0.6 SD); and, the biomarkers that showed the most predicted improvement when reducing sitting to desirable levels (25) without large changes to stepping (i.e., lipids, blood pressure, insulin, waist circumference and body fat).

Consistent with our findings, the underlying biological mechanisms would also tend to suggest that both standing and stepping should be beneficial, but with the greatest benefit for stepping. The added benefit for glycemic control associated with transitions to stepping compared with transitions to standing may reflect greater muscle and/or metabolic activity in general (27, 28), or the comparatively higher energy demand associated with activation of fast-twitch glycolytic fibres (29, 30). This contrasts with the lesser glycemic benefit of transitions to standing which involve a comparatively lower energy requirement and engagement of oxidative fibres, favoring fat metabolism (29, 30). Broadly, the findings aligned with recent acute experimental studies in

overweight adults that have sometimes indicated greater improvements in postprandial glucose and insulin responses (4, 31, 32) by interrupting sitting with intermittent ambulation compared with standing breaks. Similarly, cross-sectional isotemporal analyses have also showed stronger effects on a range of cardio-metabolic biomarkers when sitting time is reallocated to additional stepping rather than standing (26). Notably "stepping" is an amalgamation of various ambulatory activities, and the stepping findings are therefore reflective of the "~~average~~typical mix" of the various ambulatory activities that were performed by the participants of the *Stand Up Victoria* intervention, which had a predominant focus on light-intensity activity. Within the stepping category, effects of running are likely greater than walking slowly, for example. Similarly, effects of sitting are reflective of the "typical mix" of sitting for this population; it is possible that certain types of sitting (e.g., sitting in long bouts, sitting after lunch) are more deleterious than others.

Strengths of the study include the evaluation of the short- and long-term effects on objectively assessed biomarkers alongside accurately and objectively measured behaviors, with good study retention especially in the short term. A novel element was that this intervention that targeted whole-of-day behavior changes was examined with analytic methods suited to such data. A key limitation was that the study was not powered a priori for this secondary analysis and showed evidence of limited power and precision (e.g., the wide margins of error around predicted mean values). We did not adjust for co-occurring changes in the intervention (e.g., in dietary intake) as these are potentially attributable to the intervention; however, the changes may have been coincidental, and therefore our results may be subject to confounding. It appeared unlikely that the findings were strongly affected by unexamined activities or variation in total waking hours.

However, this is impossible to verify without accurate and detailed measures (e.g., high-quality sleep, time in bed unable to sleep etc.) or knowledge of activity during unobserved time. Another limitation was that the study took neither measures of post-prandial metabolism nor continuous biomarker measurements in the behavior setting (e.g., by continuous glucose monitoring or 24-hour ambulatory blood pressure monitoring). A focus on the postprandial state may be especially important for interventions targeting not only whole-of-day changes but also workplace changes, since the postprandial periods after lunch and other meals are often spent at work. Generalizability is limited, as participants were recruited non-randomly from a single organization and there was some evidence of a tendency to disproportionately lose women to follow-up. Also, our sample was a general population of workers; effects may also differ within clinical populations.

In conclusion, our study provides further insights into the heterogeneous findings of studies examining the cardio-metabolic benefits of reducing sitting time. Firstly, long-term intervention seems necessary to identify relevant changes. Secondly, if using primarily sitting-standing substitutions, these seemingly need to be large volume, and achieved without adversely impacting stepping. Finally, sitting should be replaced with ambulatory activity if benefits to fasting insulin levels are desired and for potentially greater benefits to other biomarkers as well.

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Figure 1: Change in the composition of the waking day between baseline and 12 months. The centre shows no change, and each corner is a complete change in the activity (from 0% to 100% of the waking day).

Figure 2: Predicted improvement in overall cardio-metabolic risk score (a, left) and insulin (b, right) by changes in the waking day's composition (12 months).

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